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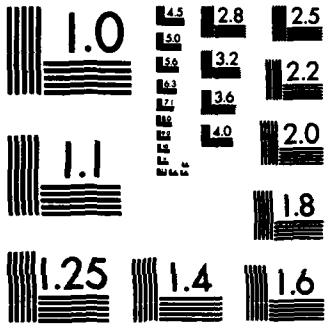

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EVALUATION STUDIES OF THE DEN-2/S-1 VACCINE

Annual Report

Edmundo Kraiselburd, Ph.D.

August 1980

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Rhesus monkeys; Dengue Type 2 live-virus Vaccine; infection enhancing antibodies.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Four groups of six nonimmune male rhesus monkeys were inoculated subcutaneously with formulations of dengue type 2 vaccine virus DEN-2/S-1. Group A received 1.9×10^4 plaque-forming units of vaccine in normal human serum albumin diluent. Group B received the same dose combined with a dengue type 2-immune human serum diluted 1:1,600, beyound its neutralization endpoint of 1:300, but having an immune enhancement titer of 250,000. Groups C and D received 10-fold dilutions of these respective formulations. No migration-inhibitory factor was found when

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20. Abstract (continuation)

peripheral blood mononuclear leukocytes obtained on day 68 post-immunization from monkeys of all experimental groups were tested. No viremia was detected in any of the monkeys when sera taken on postvaccination days 1 through 12 were inoculated into adult Toxorhynchites amboinensis mosquitoes and LLC-MK_2 cells. By day 89, four of the six monkeys had seroconverted by the neutralization test in each of groups A,B, and C, and three of five monkeys in group D (one monkey died from cardiac collapse after anesthesia) had seroconverted. Immune enhancement of dengue virus infection is known to occur in humans and monkeys circulating heterologous flavivirus antibodies. In this study, there was no enhancing effect when antibody was mixed with dengue type 2 vaccine virus and injected subcutaneously.

Infection and Immunity, 33, 389-394, 1981.

EVALUATION STUDIES OF THE DEN-2/S-1 VACCINE

In comparative studies with human volunteers, six of six yellow fever-immune but only three of five flavivirus-nonimmune subjects showed a dengue type 2 virus-neutralizing antibody response after subcutaneous inoculation of the undiluted DEN-2/S-1 vaccine. This more successful immunization of yellow fever-immune volunteers may have been due to the formation of (non-N) flavivirus-antibody complexes, which could infect more cells via Fc receptors. The experimental work presented herein represents our attempt to investigate whether immune complexes composed of nonneutralizing dengue virus antibodies and dengue virus would effect more seroconversions than would the vaccine alone when inoculated into experimental animals. This report describes the resulting humoral response induced by subcutaneous inoculation of the experimental vaccine alone and those induced by inoculation of vaccine virus-antibody complexes in experimental rhesus monkeys (Macaca mulatta).

This contract started March 1, 1980. The annual progress report represents the research work performed from April 1 to August 1 (4 months). The Dengue Virus Laboratory was officially inaugurated on June 5, 1980 by Dr. Renato Dulbecco (1975 Nobel Laureate in Medicine), Dr. Mike Bishop (Professor, Dept. of Microbiology, University of California at San Francisco), Dr. Arnold Levine (Chairman, Dept. of Microbiology, SUNY at Stony Brook), Dr. Norman Maldonado (Chancellor, UPR School of Medicine) and other local administrative officials from the UPR and the Health Department.

Actual experiment work began on April 15, 1980. Equipment (tissue culture hood and incubator) arrived in Puerto Rico during the months of April and May. A groupof 24 seronegative rhesus monkeys were selected and divided into 4 groups of 6 monkeys each. Group A monkeys received undiluted vaccine, group B monkeys received undiluted vaccine with antibody, group C one tenth diluted vaccine and group D diluted vaccine with antibody. Four additional monkeys were used as

controls. After vaccination monkeys were bled daily for 12 consecutive days and all serum samples were assayed for viremia. The results were negative for all the experimental groups. Dengue-2 HI tests were performed on sera taken on days 16, 33, 44, 58 and 89 post inoculation. Neutralization tests were performed on sera taken on the pre-inoculation day, and on days 58 and 89 post-inoculation. All pre-inoculation sera were negative (<10). Table I shows the results obtained. By day 44 HI tests revealed 33% seroconversion in group A animals, 66% in group B, 17% in group C and 60% in group D. N tests performed on day 58 revealed 83% seroconversion in groups A and B and 100% in groups C and D. However these N.t. results should be treated with caution because only 25 plaques were observed in controls samples. This was due to the deterioration of the stock virus sent by (Walter Reed Army Institute of Research) (WRAIR). It should be pointed out that N tests were also performed by WRAIR on sera taken from groups C and D on day 58 post-inoculation. Essentially, WRAIR confirmed the results obtained in Puerto Rico. However, one monkey from each group with N titers of <20 (but >10) were shown to be negative (<10) at WRAIR. However this difference does not significantly alter the interpretation of the results. N tests performed on day 89 sera showed 33% seroconversion in groups A and B, 50% in group C and 20% in group D. Therefore, it is possible that loss of antibody titer may have occurred between days 58 and 89.

The N tests show conclusively that the addition of antibody to the vaccine has no significant enhancing effect in monkeys. Therefore, the monkey cannot be used as an experimental model for studying antibody enhancement of vaccine virus when the antibody is given subcutaneously.

Overtime Work: All personnel involved in this contract worked overtime. This is because:

1. Serological tests involve much work in tissue culture and most of them were performed on the same day.

2. Viremia studies required working hours during weekends and holidays.

3. In order to handle the administrative aspects of this project, the secretary also had to work overtime. The secretary is responsible for the time consuming processing of each job order and purchase order requisition, for preparing reports of attendance of the personnel involved in this contract in addition to typing, filing and other office work.

In order to make progress in this contract, the PI had no choice but to include an overtime clause in the contracts signed by the technician, the laboratory assistant and the secretary.

Problems:

a) Technical: (1) for N tests we used sera from unvaccinated monkeys as control sera. Incubating a one tenth dilution of this serum with a known virus concentration, we observed twice as many plaques as incubating the same virus preparation with 10% FCS in M199 medium. We have no explanation for this observation. (2) N tests were performed using the PR159 DEN-2 virus strain sent by WRAIR. In our hands, each vial contained a different virus concentration. Titrations in LLC-MK2 cells revealed titers ranging from 10^2 to 10^3 pfu/ml. The expected titer was supposed to be in the order of 10^5 pfu/ml. The deterioration of the stock virus was also confirmed by WRAIR. We have also noticed a yellowish color in some sealed ampules after thawing. This may indicate the presence of CO_2 in some of the ampules. This observation was also made in one vaccine batch.

b) Suppliers: We are not getting good service from Microbiological Associates. A purchase order made in March requesting 10 x M199, vitamines and amino acids, needed for the plaque assays although promised in ten days was not received until July 31, 1980. The export manager of M.A. is Mr. Charles Griffin. It will be much appreciated if this situation could be changed.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE I: Viremia and Antibody Responses of Rhesus Monkeys after Vaccination

Monkey No.	Group	Viremia	Reciprocal Antibody Titer								
			HI on day:						N on day :		
			Pre	16	33	44	58	89	Pre	58	89
FQ5	A	-	*	*	*	20	40	20	*	280	64
DV6	A	-	*	*	10	20	20	10	*	88	<20
GE5	A	-	*	*	*	*	*	*	*	47	*
B466	A	-	*	*	*	*	*	*	*	*	N.D.
EC7	A	-	*	*	*	*	*	*	*	16	*
DXO	A	-	*	*	*	*	*	*	*	60	10
B373	B	-	*	10	*	10	*	10	*	17	*
B374	B	-	*	10	10	*	10	10	*	40	<20
GW5	B	-	*	*	10	20	20	20	*	158	25
A983	B	-	*	20	40	20	20	20	*	50	<20
B467	B	-	*	*	*	*	*	*	*	28	18
GD5	B	-	*	*	*	*	*	*	*	*	*
B14	C	-	*	*	40	20	20	20	*	40	45
A599	C	-	*	*	*	*	*	10	*	160	26
EB6	C	-	*	*	*	*	10	320	*	90	>270
EU1	C	-	*	*	*	*	*	*	*	80	*
GZ0	C	-	*	*	*	*	*	*	*	10	*
86A	C	-	*	*	*	*	*	*	*	24	*
EA7	D	-	*	*	20	10	20	10	*	78	*
DD8	D	-	*	*	10	10	20	10	*	65	<20
GD6	D	-	*	*	*	*	*	10	*	18	*
EM7	D	-	*	*	*	*	*	10	*	14	*
EE8	D	-	*	10	10	20	20	40	*	270	33
Four Control Monkeys		-	*	*	*	*	*	*	*	*	*

*less than 10

N titers of <20 taken on day 89 are being retested.

Note: 1. N tests for day 58 and 89 were performed with PR 159 and PR 119, Puerto Rican DEN-2 virus strains, repectively.

2. One monkey of group D died on day 13 post inoculation. It had no viremia and its death was due to cardiac collapse unrelated to vaccination.

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